Conference Report

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The main themes of the Clinical Science Track B were combination therapy, the role of quantitative plasma RNA (qRNA) as a predictor of clinical outcome, and protease inhibitors. With increasing evidence of the clinical effectiveness of combination therapies and the development of a useful surrogate marker to monitor therapy, there was genuine hope for the potential to further improve and sustain clinical efficacy. However, the main questions which remain are when is the optimum time to start therapy, and with which combination.

Combination therapies

The results of two clinical endpoint studies were presented that emphasised the earlier findings of the Delta and ACTG175 trials, showing that a combination of two antiretroviral drugs reduces the risk of clinical progression, including death, compared with monotherapy. The first of these, the CPCRA007 study (MoB 291) (the reference numbers given are those in Volumes 1 and 2 of the *Abstract Book*) was similar in design to Delta, but in a more advanced population. Of the 1113 study participants, 22% were zidovudine naive at entry, the median duration of prior zidovudine use was 7 months, and the median CD4 count at baseline was $92 \times 10^6/l$. Patients were followed up for a median of 34 months. Overall there was no significant difference between the three treatment arms for the number of and time to progression to a new AIDS defining illness or death (Zdv : 242/375, Zdv + ddi : 222/366, Zdv + ddC : 232/372). However, in zidovudine naive patients there was a significant reduction in rates of disease progression or death for the combination regimens compared to zidovudine alone with the combination of zidovudine and ddI providing the greatest benefit (RR = 0·54, p < 0·01) findings consistent with other studies.

The second study, the Roche NV 14526 trial, (MoB 410, LBB6033), compared a combination of ddC and saquinavir with ddC or saquinavir monotherapy in 978 patients who had previously taken zidovudine for a median of 17 months. The median baseline CD4 count was $160–180 \times 10^6/l$, median follow-up 17 months, and 16% had had an AIDS diagnosis at entry. There was an estimated 53% reduction in progression to a new AIDS defining illness or death (RR 0·47, 95%, CI 0·33–0·67), in the combination arm versus ddC alone, and a 72% reduction in mortality (RR: 0·28, 95% CI 0·13–0·60). The total number of deaths were: ddC 28/314 (9%); saquinavir 34/318 (11%); saquinavir + ddC 9/308 (3%). There was no difference in the time to AIDS or death between the saquinavir and ddC arms. The surrogate marker changes for the combination of saquinavir and ddC were not marked (MoB 410) possibly suggesting that this regimen may not be as clinically effective as others in previously treated patients. However, it is the second trial to show the clinical efficacy of a treatment regimen involving a protease inhibitor.

A meta-analysis of four phase II trials of a combination of zidovudine and 3TC vs zidovudine alone (NUCA3001, NUCB3001, NUCB3002) or vs zidovudine and ddC (NUCA3002) involving 972 patients showed an estimated 65% reduction in progression to a new AIDS defining event for the zidovudine/3TC combination relative to the control patients (ThB 948). The full results of the phase III clinical endpoint study of 3TC (Caesar) are awaited.

Several phase II studies reported the surrogate marker changes of triple combination therapy. Updated follow-up results of the Merck Sharpe Dome sponsored study (protocol 0035) of indinavir vs zidovudine + 3TC vs zidovudine + 3TC + indinavir in patients previously treated with zidovudine showed that the marked fall in plasma qRNA and rise in CD4 count in the triple combination arm first reported earlier in the year at the *3rd Conference on Retroviruses and Opportunistic Infections* were sustained beyond 44 weeks (ThB 931). Eighty percent of patients (n = 26) on the triple combination continued to sustain undetectable levels of plasma viraemia at 36 weeks. The equivalent figure for indinavir monotherapy and zidovudine + 3TC combination were 30% and < 5% respectively.

Equally impressive were the short-term results of a non-comparative study of the combination of zidovudine, 3TC and nelfinavir (LB.B 6031). Eleven of the 12 patients who had a median CD4 count of $253 \times 10^6/l$ (range 37–557) and a mean plasma qRNA of 5·32 log copies/ml at baseline became aviraemic (< 500 RNA copies/ml) after 12 weeks of therapy with a mean reduction in log plasma qRNA of 2·62 and a median rise in CD4 count of 98 cells $\times 10^6/l$. Using a more sensitive plasma qRNA assay (lower limit of detectability < 25 copies/ml), all patients were still found to be aviraemic. Ho *et al*, using the

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results from eight of these patients presented further data on viral dynamics. They reasoned that there is a first phase of decay in suppression of plasma RNA which equates to the inhibition of virus replication in productively infected CD4 cells estimating a mean replication cycle of 1·25 days (SD 0·34) which is followed by second phase decay which equates to loss of latently infected T cells chronically infected macrophages in which there is an estimated mean viral replication cycle of 13·3 days (SD 7·9). From these estimations of viral dynamics they raised the possibility of being able to "eradicate" HIV with these potent combinations if complete suppression of viral replication could be sustained for 2–3 years, with eventual burn-out of all HIV infected cells. This remains a theoretical model and does not take into account sanctuary sites of HIV replication within the body.

This level of suppression of plasma viral RNA is not limited to combinations including only a protease inhibitor, but appears also to be achieved with a combination of zidovudine, ddI and the non-nucleoside analogue RT inhibitor, nevirapine. In a study of 152 anti-retroviral naive patients who had a baseline median CD4 count of between 340–380 × 10⁶/l, Montaner *et al* reported that more than 60% of patients on the triple combination arm had undetectable plasma qRNA levels at one year of follow-up (MoB294). Mean viral load levels at one year had returned to baseline in patients on the zidovudine + nevirapine combination, were −1·0 log copies/ml of zidovudine + ddI, and −1·42 log copies/ml for the triple combination. The investigators also reported that those patients who reported good adherence to the triple combination regimen were more likely to have achieved viral load levels below the level of detectability than those patients with poor adherence. Overall the triple combination was well tolerated with 80% completing one year on therapy compared with only 57% on the zidovudine and nevirapine combination. Of 98 patients randomised to receive nevirapine, seven discontinued treatment due to a rash.

The results of these combination trials are encouraging but it remains to be determined whether three drugs are always better than two, particularly if more potent anti-retroviral agents are used in combination, and whether these greater changes in surrogate markers are sustained past one year, and can be translated into more favourable clinical outcome.

Quantitative plasma RNA
Further data were presented on the association between plasma qRNA levels and clinical progression for both predicting the natural history of HIV infection and clinical outcome on anti-retroviral therapy. Three cohort studies reported a stronger association between baseline plasma qRNA levels than CD4 count in predicting clinical progression. The largest of these involving 1604 patients from the Multicentre Aids Cohort (WeB 410) study, in whom the mean follow-up for those who developed AIDS was 4·5 years, and those who remained AIDS free 9·6 years; showed that a baseline plasma qRNA level of > 30 000 copies/ml was associated with an 18 fold increase of risk of death compared with those who had a level of < 500 copies/ml. The median time to AIDS was greater than 10 years for those with < 500 copies/ml, 8·3 years for 3–10 000 copies/ml, 5·5 years for 10–30 000 copies/ml, and 2·8 years for > 30 000 copies/ml. There was a strong correlation between baseline plasma qRNA level and the subsequent CD4 count decline. Concern was expressed about the possible under-estimation of plasma qRNA levels from the testing of frozen plasma samples that had been stored for several years.

Similar findings were reported in the Swiss cohort (WeB 413) study and a study of haemophiliacs (MoC 322). The former reported however that in patients with CD4 counts < 50 × 10⁶/l there was no association between plasma qRNA and clinical outcome or death. The advanced level of immunodeficiency at this stage of disease is clearly more important.

Plasma viraemia was also reported to be a predictor of clinical outcome in children, with 50% of children with a qRNA level of > 1·0 × 10⁶ copies/ml at 2–3 months of age progressing to AIDS or death within 12 months, compared with 7% of children at or below this value (WeB 311).

Although the level of plasma qRNA does not tell us when to initiate therapy, the short term fall in viral load on therapy appears to be a stronger predictor of clinical outcome than the change in CD4 count. Analysis of the results from the nested virology study of the Delta Trial (MoB 292) showed that the independent predictors of clinical outcome at baseline were plasma qRNA level (per log 10 decrease RH: 0·29, 95%, CI 0·15–0·55), CD4 count (per 50 cell increase, RH 0·80, 95% CI 0·7–0·98) and SI/NSI phenotype (SI RH 2·4, 95%, CI 1·3–4·4). However, on therapy only the change in plasma qRNA at week 8 was significantly associated with favourable clinical outcome (RH 0·5, 95%, CI 0·3–0·9). Similar results were reported in the ACTG 175 and CPCRA007 (ThB 911) trials. In the latter, although a greater than one log copies/ml decrease at 6 months was significantly associated with a reduced risk of clinical progression or death (RH 0·39, 95% CI 0·21–0·72), this was not sustained at 12 months (RH 0·32, 95% CI 0·16–1·10) but clearly there was a trend. In a surrogate marker analysis of the Abbott M247 (WeB 3127) study which investigated the clinical benefit of adding ritonavir to current therapy in patients with CD4 counts < 100 × 10⁶/l, a fall in plasma qRNA of greater than one log copies/ml was associated with a 91% reduction in risk of clinical progression (RH: 0·09, 95%, CI 0·01–0·74). This compared with a change of < 0·25 log copies/ml and a CD4 count increase of > 50 × 10⁶/l an 86% risk reduction (RH 0·14, 95%, CI 0·07–0·31). This compared with < 10 × 10⁶/l cell increase.

Several questions remain to be answered and include whether long term suppression of viral load is associated with a continued reduction in risk of clinical progression, what is the optimal virological response to therapy and what is the capacity for sustained recovery of T-cell function in the presence of suppression of viral replication. Patients will remain immunocompromised and at risk of clinical disease progression if recovery of T-cell function is insufficient, particularly in those with very low CD4 counts and advanced disease. Some investigators argue that ongoing viral replication at any level is associated with CD4 cell killing and therefore the aim of therapy should be complete suppression of viral replication. Factors such as a long term toxicity of drug therapy, compliance with treatment regimens and development of drug resistance may determine the success of such a strategy. Further information is also required to determine the level of viral replication in the lymphnodes in patients on anti-retroviral therapy. One study reported levels of viral RNA in lymphoid tissue 3 logs or greater than seen in plasma (ThB 915).

Protease inhibitors

Apart from the potency of combination regimens which include a protease inhibitor, data were also presented on their safety, and on the clinical efficacy of ritonavir in advanced disease. The latter study first presented at the *3rd Conference on Retroviruses and Opportunistic Infections*, (Washington, January 1996) showed that after nine months of follow-up there continued to be a significant decrease in mortality in those randomised to receive ritonavir (13%) versus placebo (19%) RH 0·64, with a greater effect seen in patients with higher CD4 counts (MoB 411).

Mild to moderate diarrhoea was reported in 18–30% of patients taking nelflinavir (TuB2129, MoB 413) but was otherwise generally well tolerated. Severe nausea and vomiting is a problem in the first 2–3 weeks of therapy with ritonavir but may possibly be reduced with an escalating starting dose regimen (MoB 411). Nephrolithiasis in 10–11% and asymptomatic hyperbilirubinaemia in 30–50% was reported in patients taking indinavir (MoB 412, ThB 931).

A dose escalating study of saquinavir and ritonavir in 120 patients previously treated with reverse transcriptase inhibitors reported a > 2 log copies/ml reduction in plasma qRNA in 85% of patients at 6 weeks of the therapy (Th 934). The interaction between these two drugs results in markedly increased drug levels of saquinavir due to the inhibition by ritonavir of the hepatic cytochrome P450 isoenzymes. Tolerability and pharmokinetics data suggest a final drug regimen of between 400–600 mg bd of ritonavir and 400 mg bd of saquinavir.

Drug resistance

The 5th International Workshop on HIV drug resistance was held in Whistler prior to the main conference. Delegates heard that similar to 3TC, the emergence of resistance to zidovudine is suppressed by mutations conferring resistance to foscarnet (Abs 2), delavirdine (in-vitro) (Abs 8) and d-dioxolane-guanosine (Abs 7) and that the increased fidelity of reverse transcriptase of the 3TC associated 184 mutant virus is insufficient to prevent dual resistance to other drugs (Abs 4, 11). Bi-directional inhibition of resistance was, however, reported with a triple combination of zidovudine, ddI and indinavir in patients naive to anti-retroviral therapy who were randomised to receive either indinavir monotherapy or a combination of zidovudine and ddI or indinavir, ddI and zidovudine (Abs 30, ThB 932). The number of genotypic mutations to the three drugs identified in viral isolates was markedly reduced in the triple combination. Thirteen of 24 patients on indinavir monotherapy compared with 2 out of 20 on the triple combination therapy developed resistant mutations to indinavir, whilst 10 out of 16 patients on a combination of zidovudine and didanosine developed mutations to either drug compared with none of the 20 patients on the triple combination. This suggests that the greater suppression of viral replication on triple combination regimens prevents the emergence of genotypic resistant mutations to these three drugs. This is likely to be the case with other combinations where there has been sufficient inhibition of viral replication.

Genotypic resistant mutations may, however, develop in the presence of continuing suppression of plasma qRNA levels. In the Delta nested virology study the proportion of the virus population with mutations to zidovudine increased faster in the combination arm compared with zidovudine monotherapy but this represented a smaller fraction of the pretreatment plasma RNA level as the viral load remained low to 112 weeks with no zidovudine resistant virus outgrowth (Abs 37, ThB 4354). The 215 zidovudine associated mutation appeared earlier on combination therapy such that by 48 weeks over 90% of the virus population had the 215 resistant genotype compared with 20% in patients on zidovudine monotherapy. It is assumed that the sustained suppression of plasma viral RNA levels in the presence of high genotype resistance to zidovudine is due to the anti-viral activity of either ddI or ddC as there was little or no phenotypic resistance to either these two drugs observed over the study period.

Other studies presented reported that in patients treated with ritonavir phenotypic resistance arises from the selective accumulation of several mutations in a stepwise manner starting with mutations at codon 82 (Abs 31, 33) and is associated with cross resistance to indinavir. In contrast in vitro work reported that the pathway to early resistance to nelflinavir is unique (mutation at codon 30) and is different from other protease inhibitors.

The emergence of multidrug resistance to dideoxy-nucleoside analogues in patients on long-term combination therapy was described in two abstracts (Abs 39, 40). Resistance was

associated with the emergence of a mutation at codon:151 which produced reduced sensitivity to zidovudine, ddC, ddI and D4T. The presence of these mutations was reported in seven out of 24 patients who received zidovudine plus ddC or zidovudine plus ddI for greater than 22 months in one study and in four out of 116 patients who received two or more of the same nucleoside analogues for greater than 6 months. The reasons why resistance developed and the clinical relevance need to be determined.

Acute primary infection

Previous studies have suggested that zidovudine monotherapy during acute primary infection may possibly delay progression to symptomatic disease but had little impact on plasma HIV qRNA levels. The results of studies presented in Vancouver showed that combination therapy started during or soon after seroconversion markedly decreases plasma qRNA levels up to 12 months after primary infection and may influence the architecture of and levels of viral RNA in lymphnodes. In a non-randomised study investigators from Switzerland (WeB 432) reported that in 12 patients with acute primary infection treated with a combination of zidovudine and ddI plasma qRNA levels fell from 5·17 log copies/ml at baseline to 1·73 log copies/ml at 6 months and in eight of the 12 patients plasma qRNA levels became undetectable. This compared with a mean fall of 5·16 to 4·27 log copies/ml in zidovudine treated (n = 15) and 5·14 to 4·62 log copies/ml in untreated (n = 16) historical controls. Lymphnode biopsies in three of the patients treated with combination therapy showed that the architecture was preserved and viral RNA was undetectable.

In a late breaker session Markowitz *et al* (ThB 933) reported the results of a study of patients who had been treated within 90 days of primary infection with a combination of zidovudine, 3TC and ritonavir. The mean plasma qRNA levels at baseline was 90 000 copies/ml. In all patients plasma qRNA levels became undetectable (< 100 copies per ml), culture of peripheral blood mononuclear cells was negative (TCID 50/10⁶) and there was a reversal of the CD4/CD8 ratio. This study is ongoing and will investigate levels of both viral RNA and proviral DNA in lymphnodes. It remains to be seen whether therapy can be stopped with continued complete suppression of viral activity.

Opportunistic infections

Reasons for the continuing high incidence of *Pneumocystis pneumoniae* pneumonia (PCP) and toxoplasmosis despite the availability of effective primary prophylaxis regimens was examined in studies from the USA and Switzerland. In the Swiss study (ThB 113) over 80% of patients (116/145) presenting with PCP had not received primary prophylaxis and the main reasons for this were unknown HIV status, not in medical follow-up

and primary prophylaxis either declined or not offered. Similar findings were reported in the USA study (TuB 114) and in the failure of prophylaxis of toxoplasmosis (TuB 412).

A secondary finding of a trial of once weekly azithromycin for prophylaxis of *Mycobacterium avium intracellular* (MAI) was a reduction in the incidence of PCP compared with rifabutin (TuB 410). The rate per 100 person years of PCP was 22·7 for rifabutin and 10·5 for azithromycin (RH: 0·48, 95%, CI 0·3–0·70), there were no differences in standard regimens of PCP prophylaxis between the two groups. Once weekly azithromycin appears an attractive option in patients with advanced disease. Clarithromycin was more effective than rifabutin in decreasing the incidence of and delaying time to disseminated MAI infection (WeB 421). Combination of the two was more effective than rifabutin but not to clarithromycin alone but resulted in higher drug toxicity. Prophylaxis with clarithromycin was, however, associated with a 29% incidence of resistance to clarithromycin in isolates of MAI from patients who failed on prophylaxis.

Nosocomial outbreaks and multi-drug resistant tuberculosis (MDR-Tb) involving HIV infected persons was reported from Buenos Aires, London and Madrid. The investigators from Argentina reported a total of 162 patients with MDR-Tb (WeB 304). A nested study in 92 of these patients who were not statistically different from the overall cohort at baseline showed that 84% had an identical pattern of DNA fingerprints confirming the existence of nosocomial transmission.

All three of these outbreaks were reminiscent of the US experience of several years ago with non-adherence, delayed diagnosis, delayed recognition of drug resistance, delayed initiation of effective therapy and transmission within hospital to immunosuppressed persons converging together. The spectre of nosocomial MDRTB associated with HIV remains high in those institutions that do not apply these lessons of clinical practice.

In the management of cytomegalovirus (CMV) disease investigators reported that valaciclovir significantly reduced the incidence of CMV disease (11·7%) compared with both high and low dose acyclovir (17·5%) but was associated with a trend to earlier mortality (ThB 300). Follow-up data from the CPCRA study of the efficacy of oral ganciclovir for prophylaxis of CMV disease showed no survival advantage or clinical benefit (ThB 301). In contrast in a study reported by Spector *et al* in 725 patients with a median CD4 count of 22 × 10⁶/l oral ganciclovir prophylaxis was effective in preventing CMV disease in both patients who were plasma CMV DNA PCR + or − at baseline. Those PCR + were at the highest risk of developing CMV disease (58%) on placebo, compared with those patients who are PCR− and taking oral ganciclovir (1%). Studies of pre-emptive treatment for CMV disease are warranted (ThB 302).

In a randomised control study of the safety and efficacy of intravenous cidofovir for the treatment of patients with progressive CMV

retinitis showed a median time to CMV retinitis progression of 115 days with a regimen, following induction, of 5 mg/kg once every two weeks (ThB 304).

Developing countries

In a cohort study of adults in rural Uganda, Morgan *et al* (TuB 310) reported a median survival of AIDS to death of 9·5 months, previously published studies from elsewhere have reported survival of the order of 6 months. Investigators from Abidjan in the Cote d'Ivoire examined the spectrum of adult disease in hospitalised patients and compared presenting CD4 cell counts for different diagnoses (ThB 4281). Bacterial septicaemia, tuberculosis and HIV wasting syndrome were the commonest diagnoses; importantly patients were presenting with advanced immune deficiency and its associated complications and it is unlikely that many African patients are dying at high CD4 cell counts of easily treatable disease.

Del Amo *et al* (WeB 3247) and Petruckevitch *et al* (WeB 3337) presented the findings of retrospective cohort study of HIV infected Africans and a matched non-African control group resident in London, the median time to development of AIDS for the Africans in the UK being about one year. Tuberculosis was the dominant AIDS defining illness in Africans; rates of late disease such as CMV and AIDS dementia complex were broadly similar in the two groups although rare in studies in Africa. CD4 cell decline was similar in the two groups but survival in Africans with AIDS was slightly more favourable (median 26 months versus 20 months) this difference was not significant after adjusting for age, sex, exposure group, year of developing AIDS and presence of TB (RH for Africans 0·9, 95% CI 0·46 to 1·77).

These findings suggest shorter survival with AIDS in Africa is unlikely to result from inherent differences in natural history or from differences between sub-types but are from lack of access to care and from exposure to acute infections prevalent in a poor environment.